XX. *Contributions to Vegetable Embryology, from Observations on the Origin and Development of the Embryo in* Tropæolum majus. *By* Herbert Giraud, *M.D., F.B.S.E., &c. Communicated by the Secretary.*

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In a paper published in the 'Annals of Natural History' (June 1840), I pointed out the state of the inquiry regarding the origin and development of the Vegetable Embryo, by presenting a view of the late researches which have been conducted on the Continent with reference to this subject; and I there showed that, from the conflicting nature of the results obtained by the observations of Schleiden* and Wydler†, and of Mirbel and Spach‡, taken in conjunction with certain morphological considerations, our opinions regarding the origin of the Embryo were still indeterminate; and that it yet remained for further observations and inquiries to establish a true theory of phanerogamic embryogeny. In a recent work by Auguste de Saint-Hilaire§, this subject is fully and most ably discussed; and after presenting a fair statement of the late observations of Schleiden and of Mirbel, and after showing the opposite views to which these observers would respectively conduct us, this author concludes by thus pointing out the necessity of further investigation: "Espérons que de nouvelles recherches achèveront de lever les doutes que l'on pourrait concevoir encore." The importance of forming a determinate conclusion regarding the origin of the embryo is greatly enhanced by the influence it must have in determining our notions of the nature and en-

* Schleiden, 'Sur la Formation de l'Ovule, et l'Origine de l'Embryon dans les Phanérogames.' Ann. des Sci. Nat., 2nde Série, Botan. Mars, 1839.

† Wydler, 'Note sur la Formation de l'Embryon.' (Extrait d'une lettre de M. Wydler, professeur à Berne, communiqué par M. A. de St. Hilaire à l'Académie des Sciences à Paris. Oct. 1838.)

‡ 'Notes pour servir à l'Histoire de l'Embryologie Végétale. Par MM. Mirbel et Spach.' Ann. des Sciences Nat., 2nde Série, Botan. Avril, 1839.

§ 'Leçons de Botanique, comprenant principalement la Morphologie Végétale. Par Auguste de St. Hilaire.' Paris 1841.

dowments of the sexual organs of plants, and of the offices which the two sets of generative organs respectively perform.

With the hope of removing some of the uncertainties which are still attached to this litigated question, and of gaining some determinate information on yet unsettled points, I have attempted a series of observations on the mode of development of the embryo in *Tropæolum majus*, the results of which will, I trust, contribute in some degree to furnish materials for a theory of phanerogamic reproduction.

The extreme simplicity of the ovarium of the *Tropæoleæ*, and the comparatively large size of their solitary ovules, render the individuals of this family peculiarly fitted for the kind of observations herein detailed; and in these respects their allies, the *Geraniaceæ*, are similarly circumstanced. The following are the essential characters of the so-called female organs of *Tropæolum majus*: "*Ovary* 1, 3-cornered, made up of 3 carpels; *style* 1; *stigmas* 3, acute; *ovules* solitary, pendulous; *fruit* indehiscent, separable into three pieces from a common axis; *seeds* large, without albumen, filling the cavity in which they lie; *embryo* large; *cotyledons* 2, straight, thick, consolidated together into a single body; *radicle* lying within projections of the cotyledons."

The following observations are arranged under seven general heads, corresponding with as many progressive periods in the growth of the so-called female organs, extending from the completion of the anatropous development of the ovule, to the perfect formation of the embryo; or from the commencement of the expansion of the bud, to the complete formation of the fruit*.

FIRST PERIOD.—On making a section of a carpel (just before the expansion of the bud), from its dorsum inwards towards the axis of the pistil, and in the direction of that axis, the solitary ovule is at the same time divided, and is found to have completed its anatropous development (Tab. XVI. fig. 1.). Continuous with that part of the columella which forms the placenta, is a portion of rather firm and dense cellular tissue, inclosing a bundle of vessels, and forming the so-called *umbilicus*: this, with the vessels it incloses, descends in apposition with the placenta to form the raphe (fig. 1, *a*.): and, near the point where it terminates in the base of the ovule, the vessels are gradually lost, or rather terminate in closed extremities. The nucleus has only one tegumentary

* The results, as here detailed, are collected from a great number of dissections.

membrane (*primine*?), at the apex of which is presented the exostome, or micropyle (fig. 1, *b.*), opening close by, and to the outside of the umbilicus: so that the direction of the nucleus is exactly parallel with that of the axis of the pistil. The conducting tissue of the style may be traced between the columella (fig. 1, *c.*) and that prolongation of the carpellary leaf which forms the style (*d.*), into the carpellary cavity, as far as the exostome, with which it is brought in contact by the anatropous development of the ovule. The vessels which proceed along the placenta to form the raphe, are spiral vessels and annular ducts; and at the point at which they make a turn downwards towards the chalaza, many of them end in closed extremities (*e.*), while the vascular structure of the raphe usually terminates in a single vessel. These vessels, together with an analogous set which run along the dorsum of the carpel, proceed from a larger bundle of vessels, which in the receptacle bifurcates into these two sets.

SECOND PERIOD.—During the expansion of the bud, before the dehiscence of the anther, and therefore before impregnation, a small elliptical cavity (fig. 2, *f.*) appears near the apex of the nucleus, having a delicate lining membrane formed by the walls of the surrounding cells. This cavity is the embryo-sac ("*sac embryonnaire*," Brongniart and F. G. F. Meyen; "*membrana amnii*," Malpighi; "*quintine*," Mirbel). From the exostome a minute canal (*g.*) may be traced in the apex of the nucleus, leading to the embryo-sac. The apex of the embryo-sac incloses, at this period, a quantity of organizable mucilage, containing many minute bodies having the appearance and character of *cytoblasts* (Schleiden) (*h.*).

THIRD PERIOD.—The apex of the nucleus, and of its tegumentary membrane, is now inclined and approximated towards the axis of the pistil. The embryo-sac is much enlarged and lengthened; its mucilage has disappeared; and in its place there is formed an elongated diaphanous utricle (fig. 3, *h.*) (*primary utricle*; "*utricule primordiale*," Mirbel; "*vésicule embryonnaire*," F. G. F. Meyen; "*l'extremité antérieure du boyau pollinique*," Schleiden) containing a quantity of globular matter ("*globulo-cellular cambium*," Mirbel; "*cytoblasts*," Schleiden). This *primary utricle* is developed wholly within the embryo-sac, from which it can be clearly seen to be distinct.

FOURTH PERIOD (after impregnation has occurred).—The pollen tubes do not

extend into the carpellary cavity; but the fovilla, with its granules, is found abundantly in the passage leading from the style to the exostome (fig. 4, *i.*)*. With the increased development of the embryo-sac, the primary utricle, as it elongates, becomes distinctly cellular, by the development of minute cells in its interior, while at the extremity, next the base of the nucleus, it is terminated by a spherical extremity, consisting of numerous globular cells (*k.*). The primary utricle, at this period, assumes the character of the *suspensor* (Mirbel); and its spherical extremity constitutes the first trace of the embryo.

FIFTH PERIOD.—At this stage the apex of the nucleus, with that of its tegumentary membrane, becomes directed more towards the axis of the pistil. The spherical extremity of the suspensor enlarges, and almost entirely fills the cavity of the embryo-sac; and it now becomes more evident that it constitutes the axis of the embryo (fig. 5, *k.*). The suspensor (*h.*) is, in a corresponding degree, lengthened by an increase in the number and size of its cells; while its upper extremity has now protruded through the apex of the embryo-sac, the apex of the nucleus, and through the micropyle. From this extremity there is a considerable development of cells, many of which (*l.*) hang loosely in the passage leading to the conducting tissue of the style, while others unite in forming a process which passes round the outside of the ovule into the carpellary cavity, and between the inner surface of the carpel and the outer surface of the ovule (*m.*). This process of cellular tissue is composed of from nine to twelve rows of cells; its extremity resembles, in appearance and in the anatomical condition of its cells, the spongiole of a root. When the ovule is removed from its carpel, and slight traction is made upon this cellular process, the suspensor, with the embryo, may be withdrawn from the embryo-sac, through the exostome and apex of the nucleus (fig. 6.); thus proving the perfect continuity of this cellular process with the suspensor, and through it with the embryo itself.

SIXTH PERIOD.—The suspensor is now more attenuated, consisting only, as

* Many other instances might be adduced in which the pollen tubes have not been found to penetrate so far as the micropyle. L. C. Treviranus mentions that there are whole families of plants in which he has never been able to discover any pollen tubes at all; and F. G. F. Meyen has never been able to trace them in *Urtica urens*.

at first, of two rows of cells; the cellular process, with which it is organically united, has reached the base of the ovule; the cells of its extremity abound in cytoblasts, showing it to be yet progressing in its development. With the increased growth of the embryo two lateral processes are observed proceeding, on opposite sides, from the axis, and evidently forming the first traces of the cotyledons (fig. 7, *k.*).

SEVENTH PERIOD.—All distinction between the nucleus and its tegumentary membrane ceases, as they are now united in one envelope inclosing the embryo-sac. The cellular process connected with the suspensor has become so much developed, that its extremity has passed around the base of the ovule, and is directed towards the axis of the pistil. The lateral processes of the axis of the embryo have become distinct fleshy cotyledons (fig. 8, *n, n.*), extending backwards from their point of origin towards the radicle, as well as forwards in the direction of the plumule; both which organs they inclose in corresponding depressions in their opposed surfaces. With the development of the radicle (*o.*) towards the exostome, the opposite extremity of the axis of the embryo (in the form of the plumule) (*p.*) extends towards the base of the nucleus, but is still inclosed in the depression formed in the concavity of the cotyledons.

The subsequent changes consist chiefly in the great development of the cotyledons, which ultimately come to occupy the whole cavity of the nucleus, filling the space usually taken up by the albumen.

The physiological inferences deducible from the foregoing statements are of great interest, as contributing to the determination of many unsettled points involved in the theory of vegetable embryogeny, and also as serving to elucidate many obscurities relating to the morphology of the embryo.

It has been shown above, that the formation of the embryo-sac, and the development of cytoblasts within it, takes place at a period prior to the impregnation of the pistil; and that even the primary utricle itself makes its appearance before the emission of the pollen from the anther, and before the expansion of the stigma; so that the origin of the primary utricle must not be referred to the influence of impregnation, as has been already pointed out by Mirbel and Spach in the case of *Zea Mays*. At its first appearance, the primary utricle is seen to be quite distinct from the embryo-sac, even at its

apex, with which, however, it is brought in contact at a subsequent period, and ultimately even penetrates that membrane; so that, in this instance at least, the primary utricle cannot result from a depression or involution of the embryo-sac, as is maintained by Adolphe Brongniart.

After the expansion of the lobes of the stigma and its impregnation, the pollen-tubes may be traced in the conducting tissue of the style, but not so far as the micropyle: in the channel, however, leading to this point, the pollen-granules are found in abundance, and are doubtless brought in contact with the outer surface of the embryo-sac through the exostome and the minute canal in the apex of the nucleus.

At this period the first trace of the embryo appears in the formation of the spherical body at the inferior extremity of the primary utricle, which has now assumed the character of the suspensor (*umbilical cord*). Hence, then, we are led to consider the origin of this simple spherical body, which is ultimately transformed into the embryo, as resulting from a peculiar process of nutrition, determined by the material or dynamic influence of the fovilla, conveyed through the medium of the primary utricle or suspensor. As it is through that organ that the embryo appears to derive its nourishment during the period of its development, we should from this function, as well as from its anatomical relations, consider the suspensor as the true umbilical cord; the medium of connexion, therefore, between the ovule and the columella (or so-called placenta) ought not to receive the name of umbilical cord or funiculus, which terms it would be well to confine to the suspensor alone; while the former might retain the appellation of *podosperm*, as referring to its relation to the ovule.

As it is necessary that an umbilical cord should be organically united with the embryo, the impropriety of considering the organ described by Malpighi in that light will become sufficiently obvious. This structure consists of a minute cellular process extending from the base of the embryo-sac to the base of the nucleus, and has been found chiefly in the *Cucurbitaceæ* and *Rosaceæ*. It appears, however, to be but a mere appendage of the embryo-sac, from which it takes its origin, and often never reaches the base of the nucleus, and therefore cannot be the medium of nutrition even to the embryo-sac. To this organ, therefore, it would be better to confine the term applied to it by Du-

trochet, and name it the *hypostate*, as pointing out merely its anatomical relations.

The cellular process proceeding from the extremity of the suspensor, next the exostome, around the outer surface of the ovule into the carpellary cavity, is an organ of somewhat unusual occurrence; but from its mode of growth and structural relations, it may be inferred to be of very essential importance to the origin and development of the embryo. Now it has been recently pointed out by F. G. F. Meyen*, that in the great majority of instances the pollen-tube, after having penetrated the micropyle, is brought in contact with the apex of the embryo-sac, with which it there contracts an adhesion: from this period the changes consequent on impregnation date their commencement; and, under the direct influence of this immediate application of the fovilla to the embryo-sac, continue with uninterrupted regularity. But in the case of *Tropæolum majus*, as the pollen-tube never reaches the embryo-sac, some additional means are required to insure that influence of the fovilla on the primary utricle which is necessary for the development, at its extremity, of the spherical cellular body, which subsequently becomes the embryo. This action, then, is effected by the projection of this cellular process from the primary utricle, which, by being immersed (so to speak) in the fovilla, is made the medium for the transmission of the latter to the primary utricle, and through it to the embryo itself; for which office the structure of its extremity (so like a spongiole) renders it peculiarly fitted.

It may now be shown how far the foregoing observations bear upon the undetermined question of the origin of the embryo. That in this plant the primary utricle and the future embryo never have any structural connexion with the extremity of the pollen-tube at their first origin, or at any subsequent period of their development, is sufficiently obvious from the fact, that the pollen-tube is never brought into contact with the embryo-sac. As the primary utricle makes its appearance before impregnation has occurred, it cannot be possible that that organ has ever formed the extremity of the pollen-tube, as is believed to be the case by Schleiden and Wydler. Moreover, as the primary utricle takes its origin wholly within the embryo-sac, and at the earliest period of its formation is not in contact with that membrane, it

* F. G. F. Meyen, 'Neues System der Pflanzen-Physiologie.

eannot have been formed by the pollen-tube pressing before it a fold of the embryo-sac in its passage into the cavity of that structure, as Schleiden has maintained.

The researches of F. G. F. Meyen* sufficiently prove that the present is not a solitary exception to the mode of origin of the embryo, which Schleiden and Wydler have described; for that observer has shown, from a very extended series of researches, that in those instanees in which the pollen-tube reaches the embryo-sac, it never penetrates, nor in any way enters the cavity of that structure; but that, after it has contracted an adhesion with the outer surface of the embryo-sac, the primary utricle (*vésicule embryonnaire*, F. G. F. Meyen) takes its origin within that cavity, so that the lining membrane of the embryo-sac always intervenes between the primary utricle and the extremity of the pollen-tube. It is evident that Sehleiden and Wydler have been misled by not properly distinguishing this fact, nor being sufficiently careful to observe the relations of the primary utricle at its very first appearanee. The point at which these observers believe the pollen-tube to lose its connexion with the primary utricle, is in fact its true extremity, which never has had any organic union with that body. The intimate nature of the impregnation of those plants in which the pollen-tube is brought in contact with the embryo-sac, is essentially the same as that of *Tropæolum majus*; but, in the latter, the fovilla is applied to the embryo-sae independently of the applieation of the pollen-tube to its outer surface; and its influence on the development of the embryo is sustained through the medium of the cellular proeess extending from the suspensor or true umbilical cord. The direetion of the axis of the embryo (being opposed to that of the nucleus and its membrane) is such as would be anticipated from the fact of its commencing its development at the apex of the embryo-sae; therefore the views whieh we may entertain of the morphology of the ovule do not necessarily afford an argument in favour of the doetrines of Sehleiden and Wydler, nor in any other way affect the question of the mode of origin of the embryo.

I have notieed in the preceding observations, that the first appearance of the eotyledons is accompanied by a corresponding elongation of the axis of the embryo, owing to an extension of its globular cells, so that the eotyledons

* F. G. F. Meyen. Opus cit.

necessarily arise from that axis. Hence the opinion held by many morphological writers, that the axis results from a union of the cotyledons, or of their petioles, is proved to be without foundation; for the foregoing observations show that the lateral projections, which constitute the first traces of the cotyledons, are composed of cellular tissue developed at these points of the axis, and are therefore new formations arising from determinate points, and deriving their nourishment from the substance of the axis.

EXPLANATION OF TAB. XVI.

Fig. 1. Section of a carpel of *Tropæolum majus*, before impregnation. *a.* Raphe. *b.* Apex of the nucleus and exostome. *c.* Columella. *d.* Prolongation of the carpellary leaf contributing to the formation of the style. *e.* Closed extremities of spiral vessels and annular ducts.

2. Section made at a more advanced period. *a, b, e.* As in fig. 1. *f.* Embryo-sac. *g.* Canal leading from the apex of the nucleus to the embryo-sac. *h.* Organizable mucilage with cytoblasts.

3. Section just before impregnation. *a, b, e.* As in fig. 1. *f. g.* As in fig. 2. *h.* Primary utricle filled with cytoblasts.

4. Section immediately after impregnation. *a, b, e, f. h.* As in fig. 3. *k.* Spherical body, forming the first trace of the embryo at the extremity of the primary utricle. *i.* Fovilla, with its granules, in the canal leading from the style to the micropyle.

5. Section, showing the embryo distinctly formed. *a, e, f.* As in fig. 4. *h.* Primary utricle, now become the suspensor or umbilical cord. *k.* Embryo. *l.* Cells connected with the suspensor. *m.* Cellular process proceeding from the suspensor into the carpellary cavity.

6. Embryo, suspensor and its cellular process removed from the ovule. *h.* Suspensor. *k.* Embryo. *l.* Cells connected with the suspensor. *m.* Cellular process of the suspensor.

7. Section, after the appearance of the first traces of the cotyledons. *h.* Suspensor. *k.* Embryo, with the first appearances of the cotyledons. *l.* Cells connected with the suspensor. *m.* Cellular process of the suspensor.

Fig. 8. Section, after the development of the cotyledons. *n.* Cotyledons. *o.* Radicle. *p.* Plumule.

9. Embryo, removed from the ovule. *a.* Suspensor. *b.* Radicle. *c.* Cotyledon. *d.* Axis of the embryo. *e.* First traces of the plumular leaves. *f.* Surface left after the removal of the opposite cotyledon.

10. Cotyledon, removed from the embryo.